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BACTERIAL GRANULE MANUFACTURING METHOD AND DEVICE

This invention is related to a manufacturing method for bacterial granules appropriate for long term retention and to a device for such a method, and especially, the invention provides a manufacturing method and a device appropriate to the method for bacterial
5 cells of granular shape which reach the intestine and which are not killed off by gastric fluid, even after orally ingested such as with bifidobacteria.

Conventionally, methods have been taken which dry
10 and retain bacterial cells for retention of microorganism such as microbes or yeast. Drying makes the bacterial cells separated by the cultivation liquid so that the moisture is 10% or less through low-temperature air drying, vacuum drying or freeze drying by adding to the
15 bacterial cells starch, protein material or organic or inorganic salt. For example, the bifidobacteria, which is assumed to be insufficient within the adult intestine, can be orally ingested by mixing with food or yogurt and

there are methods which are widely available to dry the material which is not appropriate for long term retention and to mix with other food products. The Kokoku Patent S58-46293 discloses starch containing water content at 4% or less, basic mixtures for use in tablets of one or 2 or more kinds, and a method for manufacturing lemon tablets containing bifidobacteria, having mixed and made a tablet of freeze dried bifidobacteria. Kokai Patent S58-14967 discloses a method which mixes bifidobacteria and starch, freeze dries the result, thus elevating the survival rate of the bifidobacteria.

The dried bifidobacteria manufactured by the previously described method has all suitable retention properties and is fundamentally a powder. Because its contact surface area with air is large, its retention should be especially considered and when placed inside a body, as a suitable number of bacteria is killed off having been uniformly distributed and received the effect of gastric fluid and the proportion which reaches the intestine is small. In addition, there are many problems known for retention even after drying using well-known methods, using lactic bacteria and yeast bacteria as representative of granular methods and extraction methods.

The inventors fundamentally have solved the defects of the drying methods previously described, having researched the problems to make handling easy and furthermore to obtain a dried bacteria with good retention properties. They suspended the bacteria in a retention membrane forming solution so that there is no

adhesion to the bacteria's surface as with conventional drying assistants, adhering to a uniform protective membrane formed on the bacteria's surface, having reacted the bacteria with a saline solution for solidifying and then solidifying. The inventors have succeeded in developing a method and a device appropriate to the method which proceeds in the following way: first, uniformly mix the bacteria and the preservation membrane forming solution, solidifying in clumps by injecting the mixture in a saline solution used for solidifying, separate the obtained solid matter and dry, and as necessary, coat using oil and fats having melting points at or above the body temperature.

The bacteria that are used in this invention can use microbes that are used in food product or drug manufacture such as lactic acid bacterium or yeast or for manufacturing processes or for general industrial processes. Especially, the invention is appropriate for use in microbes that are orally ingested such as bifidobacteria. It is possible to cite the following bacteria: *Bifidobacterium infantis*, *Bifidobacterium longum*, and *Bifidobacterium adlecsentis*.

The previously described microbes are cultivated using natural or synthetic cultivation bases containing sugar, salt and growth elements. After cultivation, there is concentration by performing bacteria separation using normal centrifugal separation, water or physiological saline is added to the concentrated matter, followed by dilution and washing and centrifugal separation once

again. There is a difference in concentration of the bacteria, but the operation is normally repeated 2-3 times. The culture component for the obtained washed bacteria is sufficiently eliminated and because the
5 resulting bacteria are not different, the bacteria may be used and according to the case, may further be used after dehydration.

In addition, the preservation membrane forming solution which mixes with the previously described
10 bacteria combines with alginate soda, potassium alginate, pectin, metal ions such as calcium ions such as in glucommannan and contains matter forming a preservation membrane on the bacteria's surface. The invention has an adjustable moisture function such as by starch, dextrin,
15 sugar, sodium glutinate, or sodium ascorbate and when drying, it is permissible to add material which prevents the shock of rapid dehydration and oxidation. As a composition example of this kind of mixture, it is possible, as solid material, to cite the following:
20 bifidobacteria 5-10 parts, starch 10-50 parts, sodium alginate 0.1-1.0 parts, sodium glutinate 0.5-2.0 parts and sodium ascorbate 0.5-1.0 parts.

In addition, as the saline solution used for solidifying, material is used having the capability of
25 forming liquid membranes on the bacteria's surface by combining with the principal component of the preservation membrane forming solution. No substantial bacteria death occurs and neutral solutions are used of

1% at 5-25°C of such compounds as, non-harmful to humans, calcium lactate or calcium chloride.

An explanation is given using the attached drawings of an example of the following *Bifidobacterium longum*,
5 separated using the previously described outline. 1 denotes the raw material mixture tank, providing a stirrer 2 inside that gives uniform mixing proportionally of bifidobacteria, sodium alginate, starch, sodium ascorbate, and sodium glutinate. At this time, the
10 viscosity of the mixture A is normally 200 cp. The obtained mixture A is next moved to the mixture reservoir 4 by means of the pump 3. The reservoir 4 is of the sealed type and the pipe 6 and adjustable pressure valve 6' are established on the top, passing through the air
15 compressor 5 as an adjustable pressure device. The push out pressure from the reservoir 4 is set normally at an appropriate pressure, for example, 1-3 kg/cm². In addition, the stirrer 7 is provided under the reservoir 4, preventing precipitation of the bacteria and starch.
20 Furthermore, the pipe 8 is connected to a desired position of the reservoir 4 and the other end of the pipe 8 opens to multiple distribution pipes 9. Multiple nozzles 10 are mounted in the downward direction on these distribution pipes 9. The inner diameter of the nozzles
25 10 may be 1mm or less, more specifically in the range of 0.2-0.5mm. Furthermore, the bulb 11 is established on the respective nozzles and may be constructed so as to adjust the fluid amount and is able to halt spraying.

Because pressure is applied from the air compressor 5 within the reservoir 4 by the previously described construction, the mixture A is ejected from the tip of the nozzles 10 and the ejected amount can be adjusted by
5 adjustment of the pressure of the air compressor or the bulb 11 and there may be established a pump in the middle of the pipe 8 instead of the air compressor so that it is possible to adjust other spray amounts.

The solidifying liquid tank 12 is established under
10 the nozzles 10. Let B be the solidifying liquid, introducing calcium lactate or calcium chloride into 12. The overflow conduit 13 is established on one side and on the other side, it is possible to supply the solidifying liquid B by means of the solidifying liquid reservoir 14
15 and the fixed capacity pump. The liquid's surface is positioned approximately 3-10 cm or less from the lower end of the nozzles 10.

Because of this positioning, the mixture A from the nozzle 10 is ejected under normally constant conditions,
20 solidifies in a short period of time in the solidifying liquid reservoir 12, becomes granular shaped and settles. At this time, because the bacteria is uniformly suspended in a soda alginate solution, the surface of the previously described granules makes first temporary
25 contact with the calcium ions and the bacteria within from impregnation of the calcium ions and the entire body gradually solidifies from the formation of the membrane.

Ejection from the nozzle 10 may be adjusted by the opening diameter of the nozzle 10, the composition of the

mixture A, and the gap between the nozzle 10 and the liquid surface and it is possible to manufacture granules of a desired size from microgranules to large granules by increasing or decreasing the emitting amount through
5 adjustment of the previously described conditions.

When the emitting pressure is too strong, and when the separation between nozzle and liquid surface is insufficient, the mixture A cannot be made into granules and becomes connected rod-shapes. In this way, the
10 mixture A becomes solidified granules C, which do not mutually cling to one another and precipitate out.

In order to extract the previously described solidified granules C, 24-32 mesh screen is scanned in a vertical direction to the flow of the solidified liquid B,
15 along with bounding the solidifying tank 12 by the pulley 17, there is spraying and washing using water by the nozzle 19 established above the reservoir 18 on the discharge side of the screen 16. Because of this operation, the solidified granules C are washed and
20 furthermore, when the screen 16 rotates, the granules are lowered to the middle of the vessel 20 which changes direction to downward, but the adhering solidified granules C are blown out by air established on the reverse side of the side for screen 16's restoration. The
25 granules are eliminated from the air flow from the nozzle 21 and are recovered within the entire vessel 20.

The recovered solidified granules C contain quite a bit of moisture and up to this point, because of limited retention force are next dried. Drying can be by vacuum

drying, freeze drying or flow drying as with inactive gases. The moisture after drying may consist of 1-5% granules.

The granules obtained by the previously described methods have a structure, using Figure 3, which has eliminated the oil and fat layer (≡) and the surface of the bacteria (イ) and the starch (□) are connected by the calcium alginate membrane (∧). The structure has a diameter 2-3 times that of the inner diameter of the nozzle 10, and compared to bacteria and drying agents that are mechanically mixed as done conventionally shown in the figures, the coating is completely uniform and the membrane layer also is extremely thin. In addition to a remarkable increase in retention force, because of the granular shape, handling is convenient.

Next, Table 1 shows the composition of the viable cell count when the bacteria granules obtained by embodiment 1 are retained at room temperature and at 37°C and covered by aluminium foil.

20

Table 1

	Room temperature (units/g)	37°C (units/g)
Start time	5.8 x 10 ⁸	5.8 x 10 ⁸
After 1 month	5.5 x 10 ⁸	2.3 x 10 ⁸
After 2 months	5.2 x 10 ⁸	8.7 x 10 ⁷
After 3 months	4.9 x 10 ⁸	2.9 x 10 ⁷

After 6 months	3.2 x 10 ⁸	2.0 x 10 ⁷
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The granules obtained by the method of this invention can be orally ingested and may be ingested by mixing with other food products. Furthermore, the granules may be used in processes for food products or in industrial targets. After oral ingestion, there are few bacteria which are eliminated by gastric fluid because of the calcium alginate coating, and furthermore, it is desirable to coat using oil and fat the bacteria granules for reducing elimination rates. The oil and the fat used cannot be oil and fat of a low melting point which is conventionally used in coating microbes, but oil and fat with a high melting point which does not decompose at human body temperature, for example, hardened oils having melting points of 37- 43°C.

The coating of the oils and the fats can be performed using normal methods, but there is an advantage to using fluid-bed granulation coating devices. The devices are devices which spray oil and fat and coat while sending gas from below and flowing the granules and the resistance is remarkable low in the gastric fluid because of the coating and nearly all the bacteria reaches inside the intestine. This example is shown by the experimental example.

The experiment uses the fluid-bed granulation coating device flow coater FLO-5 (trademark) manufactured by the method of Embodiment 1 and the hardened oil of

melting point 40°C 800g is decomposed under 45°C for bifidobacteria 2kg and sprayed in a flow at 50°C.

The result is that the obtained coated matter assumes a condition where the oil and fat layer (≡) is formed on the granular surface of the bacteria (イ) and starch (□) and after adding to the gastric fluid (matter adjusted to pH 1.5 using hydrochloric acid containing table salt 0.2% and pepsine) which has artificially adjusted this coating matter, there is a reaction while stirring for 1-5 hours within an isothermal tank. After reacting, the bifidus viable cell count was measured by normal methods, not at pH 7.0. The results are shown in Table 2.

Table 2

Start time	Reaction time			
	1 hr.	2 hr.	3 hr.	5 hr.
4.1 x 10 ⁸	4.0 x 10 ⁸	4.3 x 10 ⁸	4.0 x 10 ⁸	4.2 x 10 ⁸

However, the bifidus viable cell count is calculated on the basis of 1g granule.

The viable cell count obtained as described above is completely independent of the substantial number of dead cells which varies within experimental error. In addition, the oil and fat membrane (≡) is eliminated by elimination fluid when reaching the small intestine and because the granules are also destroyed, much bacteria is

eliminated from the effect of the elimination fluid. The inventors made it possible to reach the large intestine where a large part of the bifidus bacteria remained because of the death of approximately 20% in an
5 experiment performed with artificial gastric fluid.

The previously described experiment was for bifidus bacteria, but the method of this invention can be used to start lactic acid bacteria or yeast bacteria and for the drying of many viable cells. Because the obtained
10 bacteria is thin and uniformly coated by a preservation membrane as with sodium alginate, there is no form destruction even after adding to neutral or acidic high moisture food products, even for preservation and little effect of the moisture within the food products. This is
15 so because the activity is maintained for long periods compared to the conventional bacteria dried matter.

Below, explanations are made using embodiments.

Embodiment 1

20 Anaerobically cultivate Bifidbacterium longum at 37°C for 18 hours using skimmed milk culture which was decomposed using Tashinase (trademark) and concentrate the bacteria by centrifugal separator after cultivation. The cultivated liquid receives added physiological saline
25 in an appropriate amount, the matter is once again centrifugally separated and washed, the washing is repeated twice to obtain viable cells (approximately 140g as solid matter) of approximately 700g per cultivation 10 l.

Add to the viable cells 700g white potato starch 200g, monosodium glutinate 20g, and sodium L-ascorbate 10g and furthermore add 200ml 2.5% solution of pre-dissolved sodium alginate and mix uniformly the entire
5 matter.

Push out the mixture solution matter continuously using 1.3 kg/cm² pressure applied on the liquid surface of the solidifying liquid reservoir containing calcium lactate of 1% using a distance of approximately 5cm from
10 a nozzle of inner diameter 0.3mm using the device shown in Figure 1. Scoop up the solidified matter using a metal mesh net screen, spray water and after washing sufficiently dehydrate. Finally freeze dry to a thickness
15 in the range of 5-10 mm on top of a metal tray. The average particle diameter of the obtained granule was approximately 0.9mm, the volume ratio 2.75 ml/g and the absorption amount using white-coloured granules with 20% moisture was 340g.

The bifidobacterium was comprised of bifidus
20 bacteria 5.8×10^8 units/g. The activity was maintained for a long time with sealed storage and the granules were desirably added to food products such as yogurt.

Embodiment 2

25 Using culture at pH 6.2 comprised of protease decomposed matter of cow whey, cultivate for 4 hours at 40°C St. thermophilus, performing centrifugal separation and washing in the same way as in Embodiment 1 to obtain

approximately 700g (approximately 150g as solid matter) of bacteria per 10 l of culture.

Mix preservative membrane forming solution and the previously described bacteria using the same method as in Embodiment 1 and freeze dry after making granules to obtain 350g of white coloured granules having an average granular form of approximately 10mm, volume ratio 2.75ml/g, and moisture 20%.

The previously described granule had the results shown in Table 3 for the retention experiments after wrapping in aluminium foil allowing no air transmission or moisture transmission, comprising a viable cell count of 1.2×10^{10} units/g.

15

Table 3

	Room temperature retention	37°C retention
Start time	1.2×10^{10} units/g	1.2×10^{10} units/g
After 3 months	9.6×10^9	7.2×10^9
After 6 months	9.5×10^9	4.2×10^9

Embodiment 3

Add to bacteria 700g of *Bifidobacterium longum* obtained by the method of Embodiment 1, white potato starch 400g, sodium glutamate 20g, sodium L-ascorbate 10g, 5% (?) pectin 100ml and mix uniformly.

Making the previously described mixture in the same way as with Embodiment 1, eject from a nozzle of inner

diameter 0.4 mm from a height of 5cm to a 1% calcium lactate solution, making granules and scan using a 32 mesh screen. After washing with water, sufficiently dehydrate and freeze dry to obtain white-coloured granules 530g with an average granular diameter of 1mm, volume ratio 3.0ml/g and moisture 20%. The bifidobacteria viable cell count within these granules was 2.0×10^8 units/g and the count was retained for a long period of time.

10

Embodiment 4

Air cultivate *Sac. Cellevioae* at 30°C using a sugar cane culture to obtain approximately 700g per 1kg of sugar cane after centrifugal separation and washing.

15

Mix the previously described bacteria with corn starch 200g and 5% pectin 1000ml and extrude from a height of 7cm from a nozzle with inner diameter 0.5mm in the same way as in Embodiment 1, manufacture granules and after scanning using a 32 mesh screen, sufficiently wash with water, dry in a vacuum to obtain approximately 450g of granules with average granule diameter 1.2 mm and moisture 5%. These granules even during long periods of retention did not lack fermentation strength.

20

25 Brief Explanation of the Drawings

Figure 1 is an explanatory drawing of the device, Figure 2 is a cross-section of the solidifying solution reservoir of Figure 1, and Figure 3 is a cross-section expanded diagram of the coated granules.

1- mixture reservoir, 4- reservoir, 6 and 8- pipe,
10-nozzle, 12- solidifying solution reservoir, 14-
solidifying solution reservoir, 16- screen, 20- vessel,
A- mixture, B-solidifying solution, C- solid granule

5 Patent Applicant
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CLAIMS

1. A manufacturing method for bacterial granules comprising:

mixing bacterial cells and preservation membrane forming solution;

5 solidifying the mixture by injecting into a saline solution used for solidifying;

extracting the obtained solidified matter and drying; and

10 coating, as necessary, using oils and fats having melting points at or above the human body.

2. The manufacturing method for bacterial granules according to Claim 1 wherein the bacterial cells are bifidobacteria.

15 3. The manufacturing method for bacterial granules according to Claim 1 wherein the mixture of the bacterial cells and the preservation membrane forming solution is, using dried weight proportions, bacterial cells, 5-10 parts, starch 10-50 parts, and sodium alginate 0.1-1.0 parts.

4. A manufacturing device for bacterial granules comprising:

a reservoir containing bacterial cells and preservation membrane forming solution;

5 a nozzle, passing through the reservoir positioned vertically downward in the downward direction;

a solidifying reservoir established so that the liquid surface is placed in the downward direction of the nozzle; and

10 a device adjusting the spray amount of the nozzle wherein the nozzle's interior diameter is 1mm or less, desirably in the range of 0.2-0.5mm and the liquid surface gap between the tip of the nozzle and the solidifying reservoir is approximately in the range of 3-
15 10cm.

Figure 1

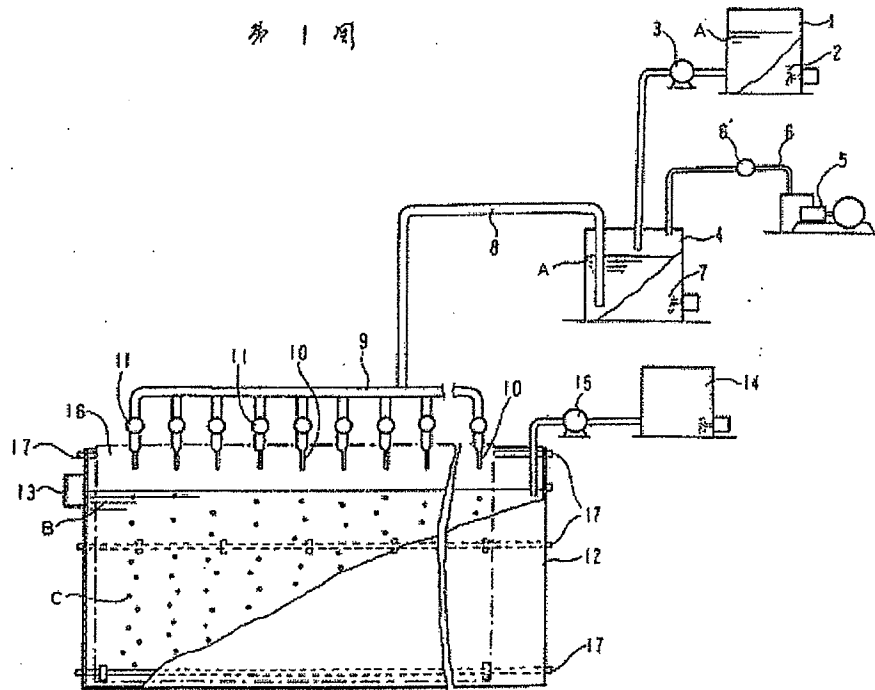


Figure 2

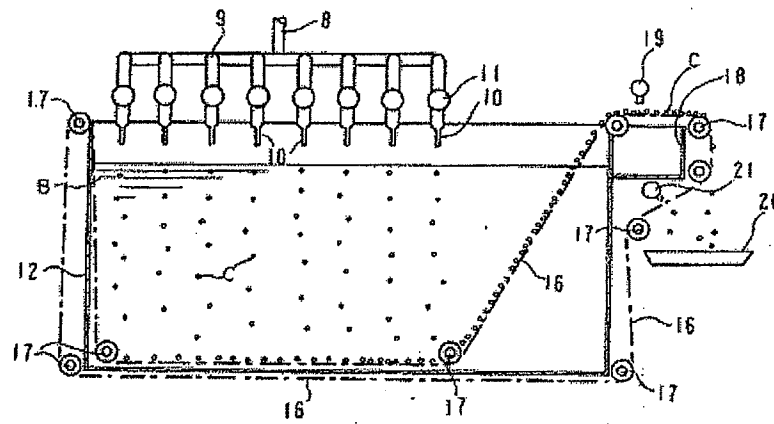


Figure 3



第 3 图